CLAIMS:

1. A novel nucleic acid probe for determining a concentration of a target nucleic acid, comprising:

a single-stranded oligonucleotide capable of hybridizing to said target nucleic acid, and

a fluorescent dye and a quencher substance, both of which are labeled on said oligonucleotide,

wherein said oligonucleotide is labeled with said fluorescent dye and said quencher substance such that an intensity of fluorescence in a hybridization reaction system increases when said nucleic acid probe is hybridized with said target nucleic acid; and said oligonucleotide forms no stem-loop structure between bases at positions where said oligonucleotide is labeled with said fluorescent dye and said quencher substance, respectively.

2. A novel nucleic acid probe according to claim 1, wherein said single-stranded oligonucleotide is labeled on the same nucleotide thereof with said fluorescent dye and said quencher substance.

3. A novel nucleic acid probe according to claim 1, wherein a distance between said bases at said positions where said oligonucleotide is labeled with said fluorescent dye and quencher substance, respectively, is 1 to 20 or {(a desired integer of from 3 to 8) + 10n} (n: an integer $\geq$ 0) in terms of the number of bases.

4. A novel nucleic acid probe according to any one of claims 1-3, wherein said single-stranded oligonucleotide has the same chain length as said target nucleic acid.

5. A nucleic acid probe for determining a concentration of a target nucleic acid, said probe being labeled with a fluorescent dye, wherein:

said probe is labeled at an end portion thereof with said fluorescent dye, and

said probe has a base sequence designed such that, when said probe hybridizes at said end portion thereof to said target nucleic acid, at least one G (guanine) base exists in a base sequence of said target nucleic acid at a position 1 to 3 bases apart from an end base of said target nucleic acid hybridized with said probe;

whereby said fluorescent dye is reduced in fluorescence emission when said probe labeled with said fluorescent dye hybridizes to said target nucleic acid.

6. A nucleic acid probe according to claim 5, wherein said probe is labeled at a 3' end thereof with said fluorescent dye.

7. A nucleic acid probe according to claim 5, wherein said probe is labeled at a 5' end thereof with said fluorescent dye.

8. A nucleic acid probe for determining a concentration of a target nucleic acid, said probe being labeled with a

fluorescent dye, wherein:

said probe is labeled at an end portion thereof with said fluorescent dye, and

said probe has a base sequence designed such that, when said probe hybridizes to said target nucleic acid, plural base pairs in a probe-nucleic acid hybrid complex form at least one G (guanine) and C (cytosine) pair at said end portion;

whereby said fluorescent dye is reduced in fluorescence emission when said probe labeled with said fluorescent dye hybridizes to said target nucleic acid.

9. A nucleic acid probe according to claim 8, wherein said probe has G or C as a 3'end base and is labeled at said 3'end thereof with said fluorescent dye.

10. A nucleic acid probe according to claim 8, wherein said probe has G or C as a 5'end base and is labeled at said 5'end thereof with said fluorescent dye.

11. A nucleic acid probe according to claim 7 or 10, wherein a hydroxyl group on a 3'carbon of ribose or deoxyribose at said 3'end or a hydroxyl group on a 3' or 2'carbon of ribose at said 3'end has been phosphorylated.

12. A nucleic acid probe according to any one of claims 1-11, wherein said probe is labeled at a 5' end phosphate group and/or a 3' end phosphate group thereof with said fluorescent dye.

13. A nucleic acid probe for determining a concentration

of a target nucleic acid, said probe being labeled with a fluorescent dye, wherein:

said probe is labeled at a modification portion other than a 5′ end phosphate group or a 3′ end OH group thereof with said fluorescent dye, and

said probe has a base sequence designed such that, when said probe hybridizes to said target nucleic acid, plural base pairs in a probe-nucleic acid hybrid complex form at least one G (guanine) and C (cytosine) pair at said modification portion;

whereby said fluorescent dye is reduced in fluorescence emission when said probe labeled with said fluorescent dye hybridizes to said target nucleic acid.

14. A nucleic acid probe according to any one of claims 1-13, wherein said oligonucleotide of said probe is a chemically-modified nucleic acid.

15. A nucleic acid probe according to claim 14, wherein said chemically-modified nucleic acid is 2′-O-methyloligonucleotide, 2′-O-ethyloligonucleotide, 2′-O-butyloligonucleotide, 2′-O-ethyleneoligonucleotide, or 2′-O-benzyloligonucleotide.

16. A nucleic acid probe according to any one of claims 1-15, wherein said oligonucleotide of said probe is a chimeric oligonucleotide which comprises a ribonucleotide and a deoxyribonucleotide.

17. A nucleic acid probe according to claim 16, wherein

said chimeric oligonucleotide comprises 2'-O-methyloligonucleotide, 2'-O-ethyloligonucleotide, 2'-O-butyloligonucleotide, 2'-O-ethyleneoligonucleotide, or 2'-O-benzyloligonucleotide.

18. A method for determining a concentration of a target nucleic acid, which comprises:

hybridizing a nucleic acid probe according to any one of claims 1-17 to said target nucleic acid, and

measuring an intensity of fluorescence in a measuring system.

19. A method for determining a concentration of a target nucleic acid, which comprises:

hybridizing a nucleic acid probe according to any one of claims 1-17 to said target nucleic acid, and

measuring a change in fluorescence emission from said fluorescent dye after said hybridization relative to fluorescence emission from said fluorescent dye before said hybridization.

20. A method for determining a concentration of a target nucleic acid by using a nucleic acid probe according to any one of claims 14-17, wherein said nucleic acid probe and said target nucleic acid are hybridized to each other after subjecting said target nucleic acid to heat treatment under conditions suited for sufficient degradation of a high-order structure of said target nucleic acid.

21. A method according to claim 20 for determining a concentration of a target nucleic acid, wherein a helper probe for the practice of a hybridization reaction is added to a hybridization reaction system before said hybridization reaction.

22. A method for analyzing or determining polymorphism and/or mutation of a target nucleic acid, which comprises:

hybridizing a nucleic acid probe according to any one of claims 1-17 to said target nucleic acid, and

measuring a change in an intensity of fluorescence.

23. A novel quantitative, polymorphous analysis method comprising:

amplifying a target gene by a quantitative gene amplification method; and

performing a polymorphous analysis with respect to said target gene to determine an amount of said target gene and a polymorphous composition or amounts of individual components of said target gene.

24. A quantitative, polymorphous analysis method according to claim 23, wherein said polymorphous analysis is T-RELP (terminal restriction fragment length polymorphism), RFLP (restriction fragment length polymorphism), SSCP (single strand conformation) or CFLP (cleavage fragment length polymorphism).

25. A quantitative, polymorphous analysis method

according to claim 23 or 24, wherein said quantitative gene amplification method is quantitative PCR.

26. A quantitative, polymorphous analysis method according to claim 25, wherein said quantitative PCR is conducted using a nucleic acid probe according to any one of claims 1-17.

27. A quantitative, polymorphous analysis method according to claim 25, wherein in said quantitative PCR, a nucleic acid probe according to any one of claims 1-17 is used as a primer, and a change in fluorescence emission from said fluorescent dye is measured.

28. A quantitative, polymorphous analysis method according to any one of claims 23-27, wherein said quantitative PCR is real-time monitoring quantitative PCR.

29. A kit for determining a concentration of a target nucleic acid, wherein said kit includes or is accompanied by a nucleic acid probe according to any one of claims 1-17.

30. A kit according to claim 29 for determining a concentration of a target nucleic acid, wherein said kit includes or is accompanied by a helper probe.

31. A kit for analyzing or determining polymorphism and/or mutation of a target nucleic acid, comprising a nucleic acid probe according to any one of claims 1-17.

32. A kit according to claim 31 for analyzing or determining polymorphism and/or mutation of a target nucleic

acid, which includes or is accompanied by a helper probe.

33. A reagent kit for use in quantitative PCR in a quantitative, polymorphous analysis method according to any one of claims 23-28, wherein said kit includes or is accompanied by a nucleic acid probe according to any one of claims 1-17.

34. A device for determining a concentration of at least one target nucleic acid out of plural nucleic acids, comprising:

a solid support, and

a like plural number of nucleic acid probes as defined in any one of claims 1-17 bound on a surface of said solid support such that said concentration of said target nucleic acid can be determined by hybridizing said target nucleic acid to the corresponding one of said probes and determining a change in an intensity of fluorescence.

35. A device according to claim 34, wherein said probes are arranged and bound in an arrayed pattern on said surface of said solid support.

36. A device according to claim 34 or 35, wherein said probes bound on said surface of said solid support are each independently provided with at least one temperature sensor and at least one heater arranged on an opposite surface of said solid support such that an area of said solid support, where the corresponding probe is bound, can be controlled to meet optimal temperature conditions.

37. A method for determining a concentration of at least

one target nucleic acid out of plural nucleic acids, which comprises determining said concentration of said target nucleic acid by using a device according to any one of claims 34-36.

38. A method for analyzing or determining polymorphism and/or mutation of a target nucleic acid, which comprises using a device according to any one of claims 34-36.

39. A quantitative, polymorphous analysis method, which comprises using a device according to any one of claims 34-36.

40. A method according to any one of claims 18-21 for determining a concentration of a target nucleic acid, wherein said target nucleic acid is a nucleic acid contained in cells derived from a microorganism or animal obtained by single colony isolation or a nucleic acid contained in a homogenate of said cells.

41. A method according to claim 22 for analyzing or determining polymorphism and/or mutation of a target nucleic acid, wherein said target nucleic acid is a nucleic acid contained in cells of a co-cultivation system of microorganisms or symbiotic cultivation system of microorganisms or a nucleic acid contained in a homogenate of said cells.

42. A quantitative, polymorphous analysis method according to any one of claims 23-28, wherein said target nucleic acid is a nucleic acid contained in cells of a co-cultivation system of microorganisms or symbiotic cultivation

system of microorganisms or a nucleic acid contained in a homogenate of said cells.

43. A method for determining a concentration of a target nucleic acid by using PCR, which comprises:

conducting reactions in PCR by using a nucleic acid probe according to any one of claims 1-17, and

determining an initial concentration of the amplified target nucleic acid from percentage of a change in an intensity of fluorescence occurred as a result of hybridization between said probe and said amplified target nucleic acid.

44. A method for determining a concentration of a target nucleic acid by using PCR, which comprises:

conducting reactions in PCR by using as a primer a nucleic acid probe according to any one of claims 1-17, and

determining an initial concentration of the amplified target nucleic acid from percentage of a change in an intensity of fluorescence occurred as a result of hybridization between said primer or an amplified nucleic acid amplified from said primer and said amplified target nucleic acid.

45. A method for determining an initial concentration of a target nucleic acid amplified in PCR, which comprises:

conducting reactions in PCR by using a nucleic acid probe according to any one of claims 1-17;

measuring an intensity of fluorescence in a reaction system in which in a course of a nucleic acid extending reaction,

said probe has been degraded out by polymerase or in which a nucleic acid denaturing reaction is proceeding or has been completed and also an intensity of fluorescence in said reaction system in which said target nucleic acid or amplified target nucleic acid is hybridized with said nucleic acid probe; and then

calculating percentage of a change in said latter intensity of fluorescence from said former intensity of fluorescence.

46. A method for determining an initial concentration of a nucleic acid amplified in PCR, which comprises:

conducting reactions in PCR by using, as a primer, a nucleic acid probe according to any one of claims 1-17;

measuring an intensity of fluorescence in a reaction system in which said probe and said target nucleic acid or amplified nucleic acid have not hybridized with each other and also an intensity of fluorescence in said reaction system in which said probe and said target nucleic acid or amplified nucleic acid are hybridized with each other; and then

calculating percentage of a decrease of said former intensity of fluorescence from said latter intensity of fluorescence.

47. A method according to claim 45 or 46 for determining a concentration of a nucleic acid amplified in PCR, wherein said PCR is real-time quantitative PCR.

48. A method for analyzing data obtained by a determination method according to any one of claims 18-28 or any one of claims 37-47, further comprising correcting an intensity value of fluorescence in a reaction system, said intensity value being available after said target nucleic acid has hybridized to said nucleic acid probe labeled with said fluorescent dye, in accordance with an intensity value of fluorescence in said reaction system available after a probe-nucleic acid hybrid complex so formed has been denatured.

49. A method for analyzing data obtained by a real-time quantitative PCR method according to claim 47, further comprising, as a correction processing step, correcting an intensity value of fluorescence in a reaction system, said intensity being available in each cycle after said amplified nucleic acid has conjugated to said fluorescent dye or after said amplified nucleic acid has hybridized to said nucleic acid probe labeled with said fluorescent dye, in accordance with an intensity value of fluorescence in said reaction system available after a nucleic acid-fluorescent dye conjugate or probe-nucleic acid hybrid complex so formed has been denatured in said cycle.

50. A method for analyzing data obtained by a real-time quantitative PCR according to claim 49, wherein said correction-processing step is performed in accordance with the following formula (1) or formula (2):

$$f_n = f_{hyb,n}/f_{den,n} \quad (1)$$

$$f_n = f_{den,n}/f_{hyb,n} \quad (2)$$

where

f_n: correction-processed value in an n^{th} cycle as calculated in accordance with the formula (1) or formula (2),

$f_{hyb,n}$: intensity value of fluorescence of the reaction system available after said amplified nucleic acid has conjugated to said fluorescent dye or said amplified nucleic acid has hybridized to said nucleic acid probe labeled with said fluorescent dye in said n^{th} cycle, and

$f_{den,n}$: intensity value of fluorescence of the reaction system available after said formed fluorescent dye-nucleic acid conjugate or said formed probe-nucleic acid hybrid complex has dissociated in said n^{th} cycle.

51. A method according to claim 50 for analyzing data obtained by a real-time quantitative PCR according to claim 47, which comprises:

introducing correction-processed values, which have been calculated in accordance with the formula (1) or formula (2) in individual cycles, into the following formula (3) or (4) to calculate rates or percentages of changes in fluorescence between samples in said individual cycles:

$$F_n = f_n/f_a \quad (3)$$

$$F_n = f_a/f_n \qquad (4)$$

where

F_n: rate or percentage of a change in fluorescence in an n^{th} cycle as calculated in accordance with the formula (3) or formula (4),

f_n: correction-processed value calculated in said n^{th} cycle as calculated in accordance with the formula (1) or formula (2), and

f_a: correction-processed value calculated in a given cycle before a change in f_n is observed as calculated in accordance with the formula (1) or formula (2); and

comparing said rates or percentages of changes in fluorescence.

52. A method according to claim 51 for analyzing data obtained by a real-time quantitative PCR according to claim 47, which comprises the following processing steps:

1) performing processing in accordance with the following formula (5), (6) or (7) by using data of rates or percentages of changes in fluorescence as calculated in accordance with said formula (3) or (4):

$$\log_b(F_n),\ \ln(F_n) \qquad (5)$$

$$\log_b\{(1-F_n) \times A\},\ \ln\{(1-F_n) \times A\} \qquad (6)$$

$$\log_b\{(F_n-1) \times A\},\ \ln\{(F_n-1) \times A\} \qquad (7)$$

where

A,b: desired numerical values, and

F_n: rate or percentage of a change in fluorescence in an n^{th} cycle as calculated in accordance with the formula (3) or formula (4),

2) determining a cycle in which said processed value of said processing step 1) has reached a constant value,

3) calculating a relational expression between cycle of a nucleic acid sample of a known concentration and the number of copies of said target nucleic acid at the time of initiation of a reaction, and

4) determining the number of copies of said target nucleic acid in an unknown sample upon initiation of PCR.

53. A method for analyzing a melting curve of a target nucleic acid, which comprises:

performing PCR on said target nucleic acid by using a nucleic acid probe according to any one of claims 1-17; and

analyzing said melting curve of said target nucleic acid to determine a Tm value of each amplified nucleic acid.

54. A data analyzing method for a real-time quantitative PCR method according to any one of claims 49-52, which comprises the following steps:

gradually heating a PCR-amplified nucleic acid from a low temperature until complete denaturation of said nucleic acid;

measuring an intensity of fluorescence at predetermined time intervals during said heating step;

displaying results of said measurement as a function of

time on a display such that a melting curve of said nucleic acid is drawn on said display;

differentiating said melting curve to obtain differentiated values (-dF/dT, F: intensity of fluorescence, T: time);

displaying said differentiated values as derivatives on said display; and

determining a point of inflection from said derivatives.